Diversity of ectomycorrhizas in lowland and mountain forests of South Sumatra

Bio Asia Project Workshop * : « Optimization of Land Rehabilitation and Forest Plantations through the Utilization of Symbiotic Soil Microorganisms »

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General context of the study

BIO-ASIA project : « Role of mycorrhizal fungi in the natural regeneration, sustainable management and biodiversity of Dipterocarp forests in South-East Asia

⇒ Overexploitation of forests in Southeast Asia activated by intensive cultivation of perennial crops like oil palm and rubber ;

⇒ Rapid extinction of native forests accompanied with loss of plant diversity and associated microbes ;

⇒ a) Preservation and restoration of forests, b) land rehabilitation programs and c) agroforestry practices have become priorities to ensure sustainable production of timber and soil conservation.

Objectives of this study

Natural forests in South-East Asia are dominated by ectomycorrhizal trees represented by a high diversity of species mostly from the Dipterocarpaceae and Fagaceae families :

⇒ Ectomycorrhizal fungi (ECM) are essential symbiotic partners for the conservation and regeneration of forest trees

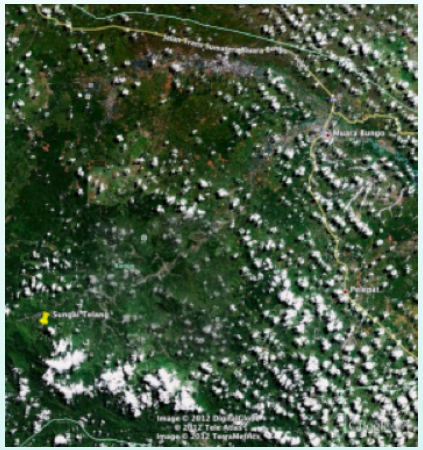
⇒ Diversity of ECM of these forests remains poorly known

- 1st objective : to describe the spatio-temporal diversity of ectomycorrhizal trees and their associated ECM fungi in two types of natural forests in South Sumatra (lowland and mountain forests)

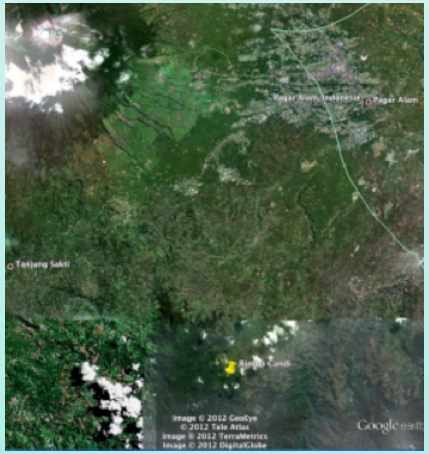
- 2nd objective : to study the role of ECM fungi in the growth, nutrition and regeneration of seedlings

Materials & Methods

- I - Plots studied: 2 forest types -> 1) Sungai Telang (Jambi prov.) Lowland Dipterocarp forest



2) Rimbo Candi (South Sumatra) Mountain forest



Materials & Methods

- I - Plots studied

2 forest types :

1) Lowland Dipterocarp forest: Sungai Telang site (Jambi prov.)

=> Natural forest dominated by Dipterocarpaceae species

-> Delineation of a 2,6 ha plot (01.69635°S; 101.78889°E, altitude : 300 m)

2) Mountain forest: Rimbo Candi site (Pagar Alam/South Sumatra)

=> Natural forest forest dominated by Fagaceae species

-> Setting up of two 50 m-length transects (04.16563°S, 103.19810°E, altitude 1,450 m)

Materials & Methods

- II – Plant and fungal material collected

1 - **Sapwood samples =>** botanical identification through anatomical observation & partial sequencing of the *trn*L chloroplast gene

2 - **Sporophores of ectomycorrhizal fungi =>** identification according to morphological traits

3 - **Ectomycorrhizal root samples =>** morphotyping under binocular microscope; storage at -80°C before dual molecular characterization of the fungus and its host plant by sequencing of the rDNA ITS region and *trn*L chloroplast gene respectively

Results

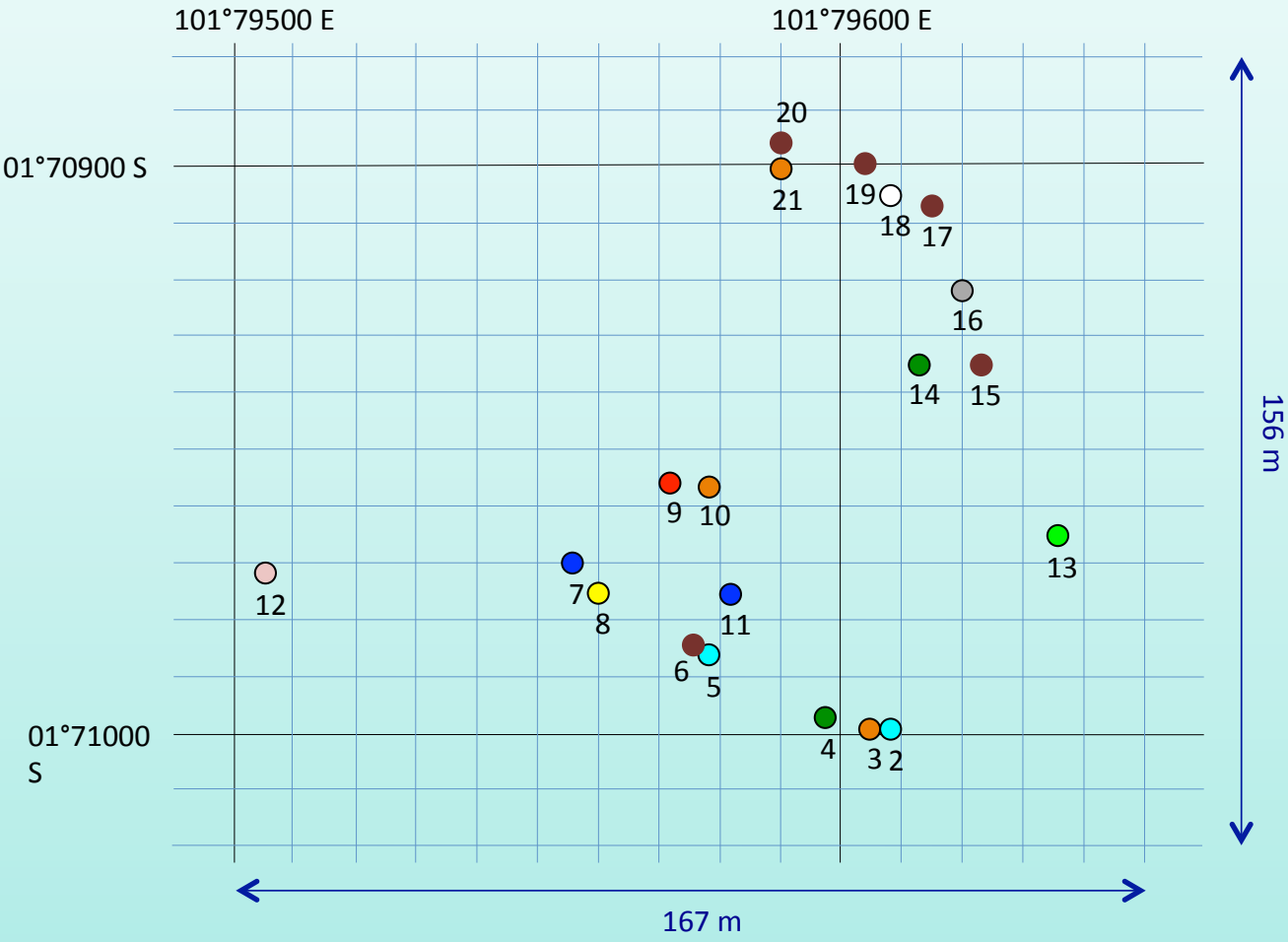
Characterization of trees

=> Delineation of plots, *in situ* identification and mapping of target trees through GPS positionning

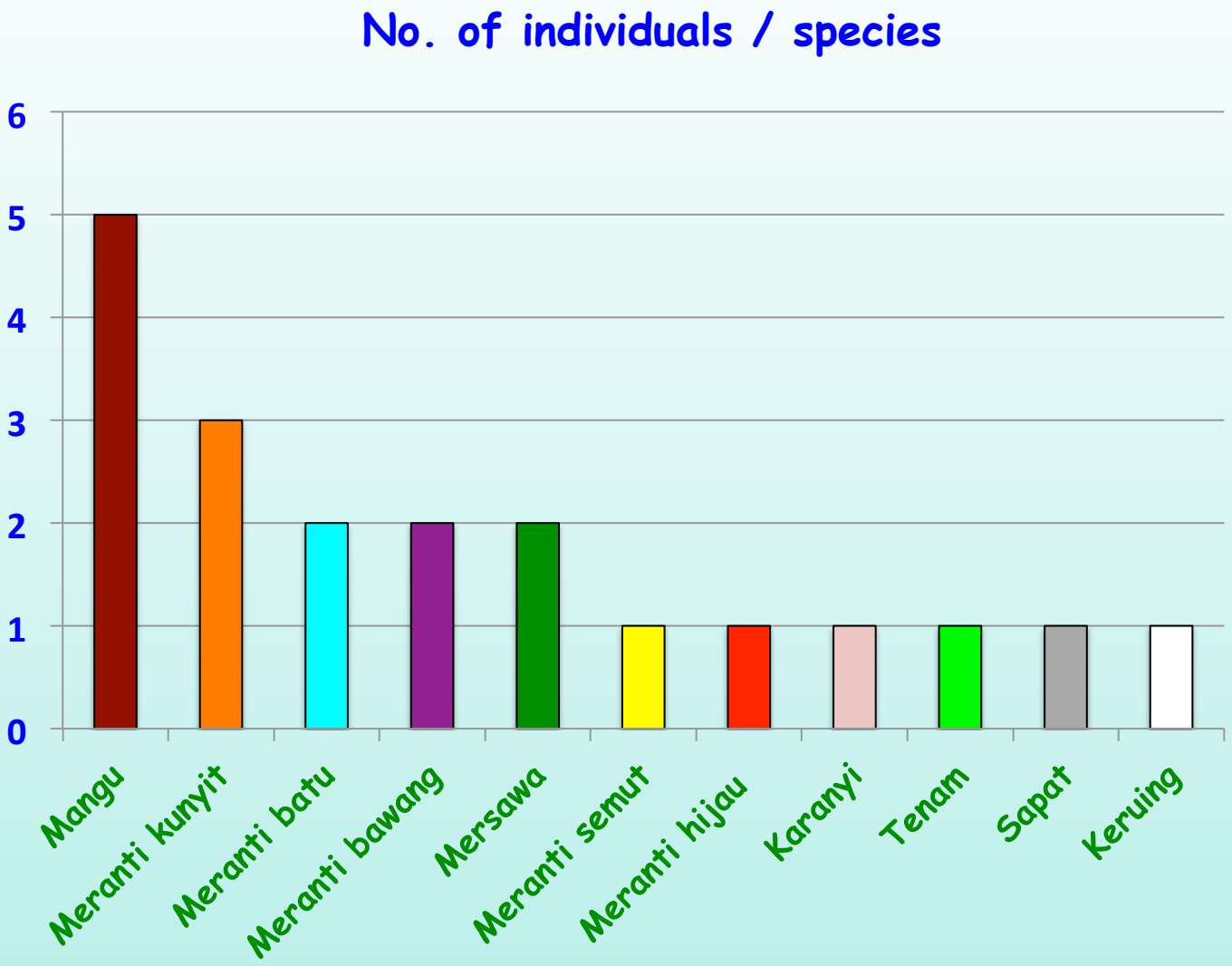
1) Lowland Dipterocarp forest: Sungai Telang (Jambi province)

=> Delineation of a 2.6 Ha

Mangu : n°6, 15, 17, 19, 20
Meranti kunyit : n°3, 10, 21
Meranti batu : n°2, 5
Meranti bawang : n°7, 11
Mersawa : n°4, 14
Meranti semut : n°8
Meranti hijau : n°9
Kranji : n°12
Tenam : n°13
Sapat : n°16
Keruing : n°18



Tree identification



20 trees from 125 to 395 cm circumference

Species name and diameter (cm) of the selected trees in Sungaï Telang (lowland Dipterocarp forest)

Site	Tree number	Species name	Diameter at 2 m height (cm)
Sungai Telang	1	*Hopea mengarawan or Shorea leprosula*	80
	2	*Shorea dasyphylla*	62
	3	*Shorea hemsleyana* (King) King ex Foxw.	42
	4	*Anisoptera* sp.	47
	5	*Shorea dasyphylla*	55
	6	*Shorea macroptera*	62
	7	*Shorea acuminata*	50
	8	*Shorea parvifolia*	48
	9	*Shorea leprosula*	58
	10	*Shorea hemsleyana* (King) King ex Foxw.	95
	11	*Shorea acuminata*	58
	12	*Dialium* spp.	131
	13	*Anisoptera costata*	42
	14	*Anisoptera* sp.	57
	15	*Shorea macroptera*	140
	16	*Shorea lepidota*	82
	17	*Shorea macroptera*	73
	18	*Dipterocarpus* sp.	65
	19	*Shorea macroptera*	63
	20	*Shorea macroptera*	58
	21	*Shorea hemsleyana* (King) King ex Foxw.	

Characterization of trees

2) Mountain forest: => Rimbo Candi (Pagar Alam-South Sumatra)

=> Delineation of 2 transects of 50 m-length x 10 m-width = 1000 m^2

Medang : n°29, 31, 32, 34, 35, 39

Tenam : n°22, 25, 26, 28, 30

Pasang : n°23, 24', 27

Kapas : n°24, 27'

Kayu Darah : n°31', 33

Medang Cabe : n°40, 44

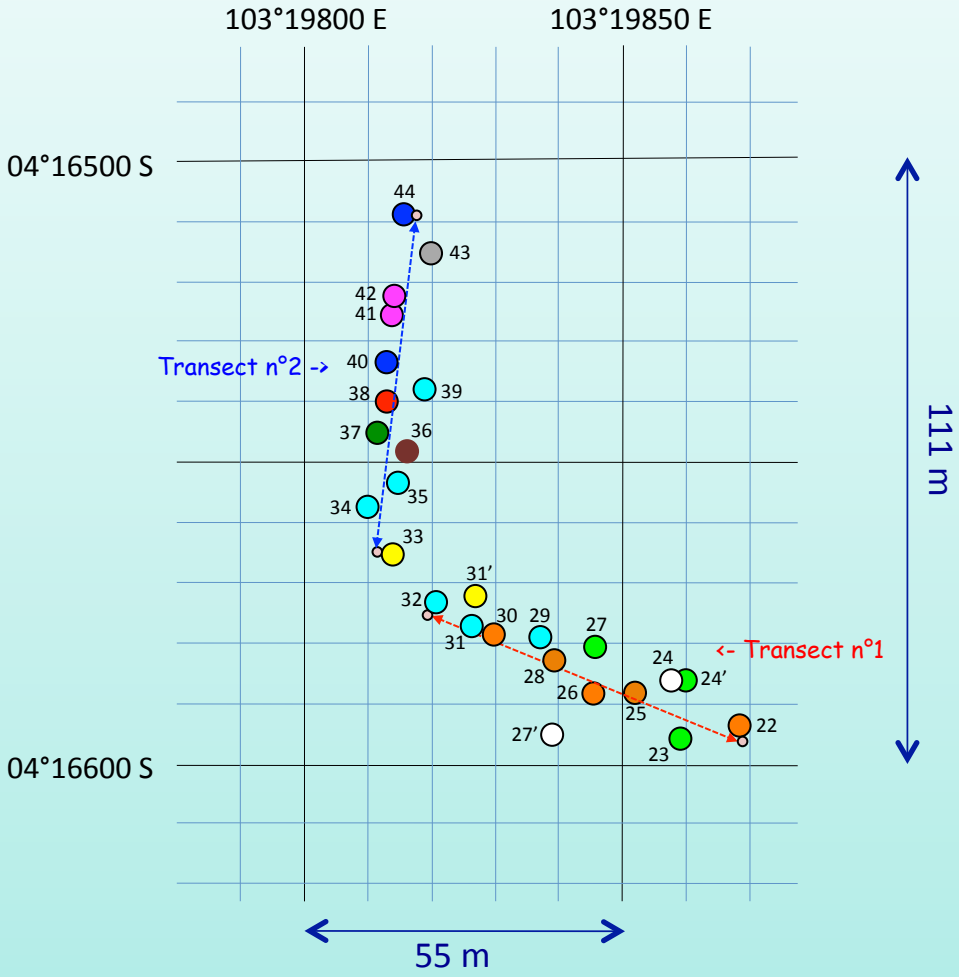
Siru : n°41, 42

Gemiling Hutan : n°36

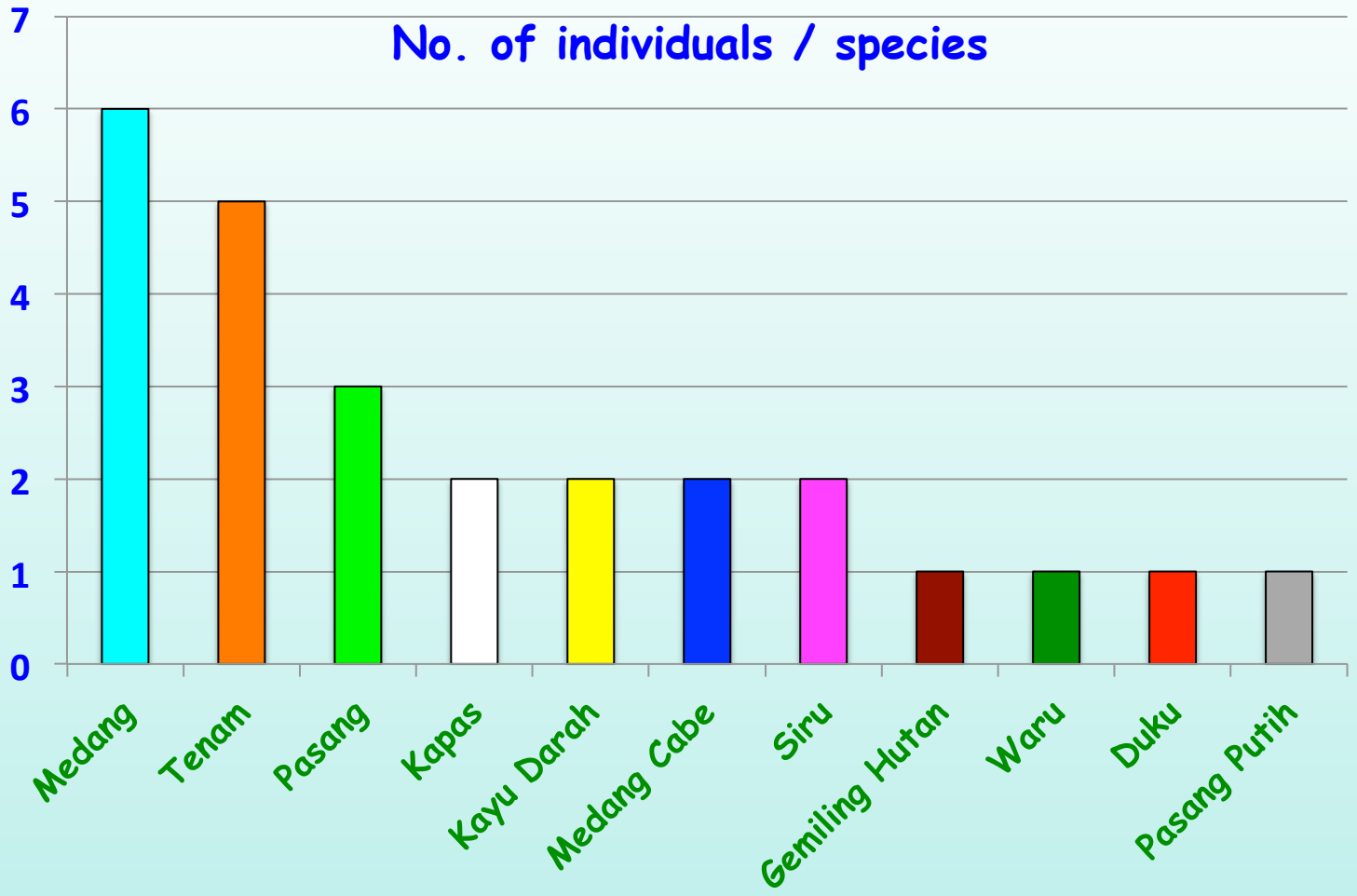
Waru : n°37

Duku : n°38

Pasang putih: n°43



Tree identification



26 trees from 40 to 150 cm circumference

Species name and diameter (cm) of the selected trees in Rimbo Candi (Mountain forest)

Rimbo Candi	22	*Zyzygium sp.*	50
	23	*Lithocarpus sundaicus*	45
	24	Undetermined sp. (Kapas)	33
	24 bis	*Lithocarpus sundaicus*	17
	25	*Zyzygium sp.*	27
	26	*Zyzygium sp.*	27
	27	*Lithocarpus sundaicus*	37
	27 bis	Undetermined sp. (Kapas)	133
	28	*Zyzygium sp.*	27
	29	*Cinnamomum parthenoxylon*	47
	30	*Zyzygium sp.*	13
	31	*Cinnamomum parthenoxylon*	25
	31 bis	*Myristica selebica*	20
	32	*Cinnamomum parthenoxylon*	18
	33	*Myristica selebica*	23
	34	*Cinnamomum parthenoxylon*	21
	35	*Cinnamomum parthenoxylon*	17
	36	Undetermined sp. (Gemiling Hutan)	15
	37	Undetermined sp. (Waru)	24
	38	*Agleia aspera*	9
	39	*Cinnamomum parthenoxylon*	17
	40	*Cinnamomum* sp.	18
	41	Undetermined sp. (Siru)	27
	42	Undetermined sp. (Siru)	17
	43	*Lithocarpus* sp.	32
	44	*Cinnamomum* sp.	18

Sporophores of Ectomycorrhizal (E) and Saprophytic (S) fungi observed at Sungai Telang and Rimbo Candi

Sungai Telang (Oct. 2011)

Russula annulata (E)
Russula cf. *xerampelina* (E)

Paneolus sp. (S)
Microporus xanthopus (S)
Microporus sp. (S)
Mycena sp. (S)
Podoscypha sp. (S)
Collybia sp. (S)
Cookenia sp. (S)
Lepiota sp. (S)
Schizophyllumcommune (S)
Mycomycete (S)
Marasmius sp. (S)
Stereum sp. (S)
Pycnoporus sanguineus (S)

-> 2 Ectomycorrhizal
+ 13 Saprophytic species


Boletus sp.


Lactarius sp1.


Russula annulata

Rimbo Candi (April 2011)

Boletus sp. (E)
Inocybe gr fastigata (E)
Lactarius sp1. (E)
Lactarius sp2. (E)
Lactarius sp3. (E)
Lactarius sp4. (E)
Russula annulata (E)
Russula virescens (E)
Russula sp1. (E)
Russula sp2. (E)
Russula sp3. (E)
Russula sp4. (E)
Russula sp5. (E)

Laetiporus sulfureus (S)
Lentinus sp. (S)
Pluteus sp. (S)
Marasmius sp. (S)
Polyporus tenuiculus (S)
Ceratomyxa fruticulosa (S)
Microporus xanthopus (S)
Ochroporus ingnarius (S)
Daldinia concentrica (S)
Stereum sp. (S)
Lycoperdon cf. *perlatum* (S)


Russula sp1.


Russula sp3.

-> 13 Ectomycorrhizal
+ 11 Saprophytic

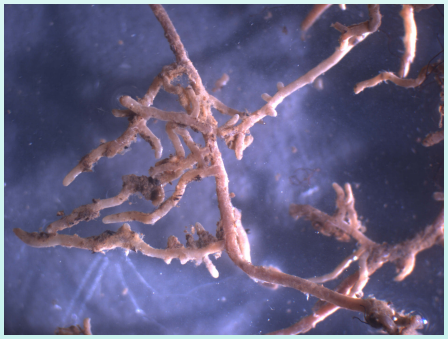
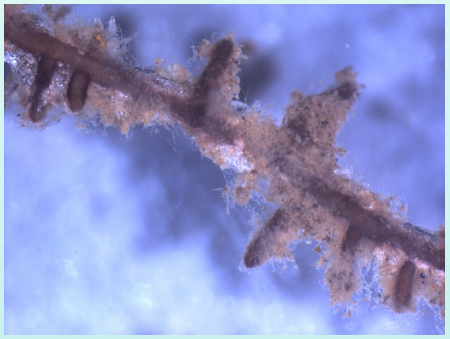

Russula sp6.


Russula sp7.

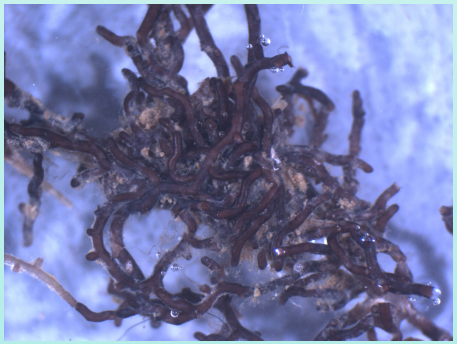
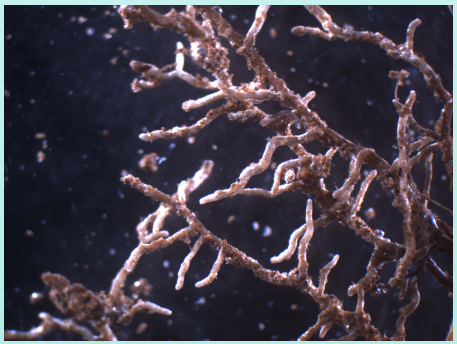
Morphotyping of ectomycorrhizal (ECM) root tips

=> ECM root tips were found on 100% of trees in Sungaï Telang site

=> Overall, about 200 different morphotypes of ECM root tips were observed and sorted for molecular analyzes



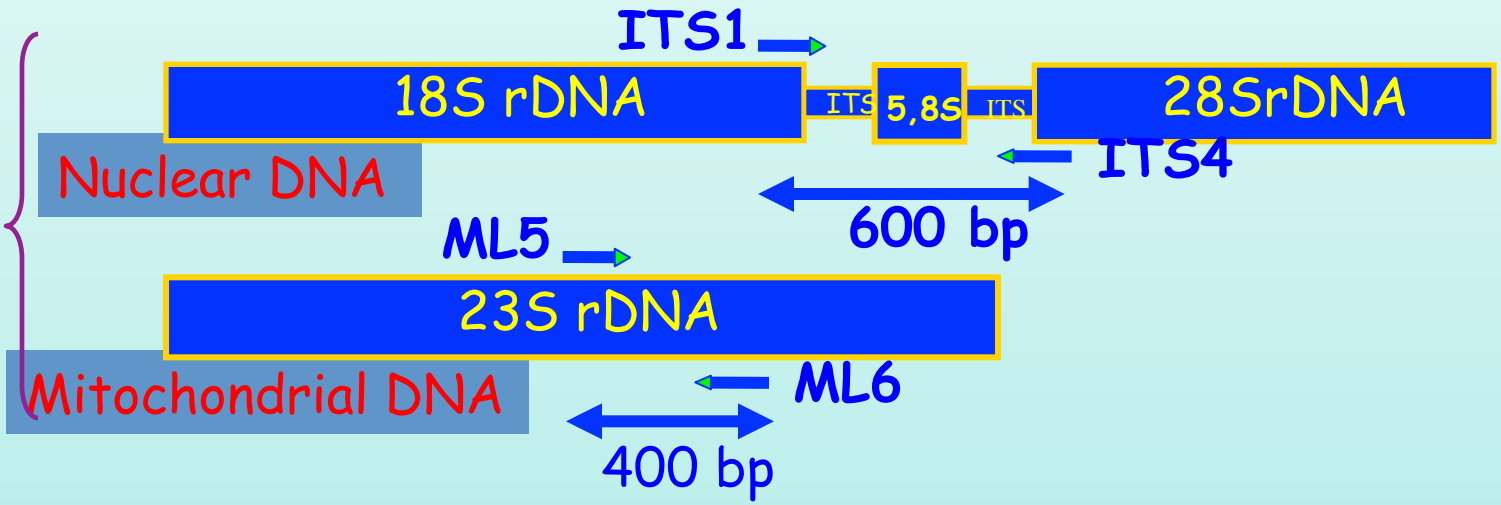

Molecular Characterization Ectomycorrhiza

DNA Extraction

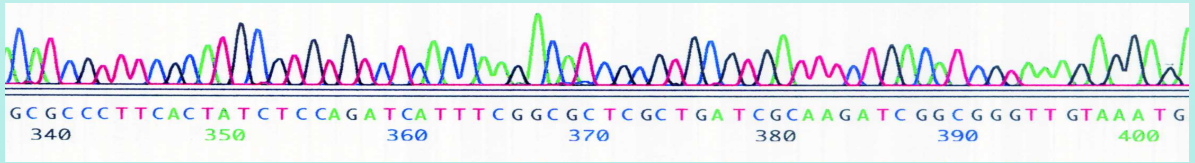
- Ectomycorrhiza
- Mycelium culture
- Sporophore fragment

PCR

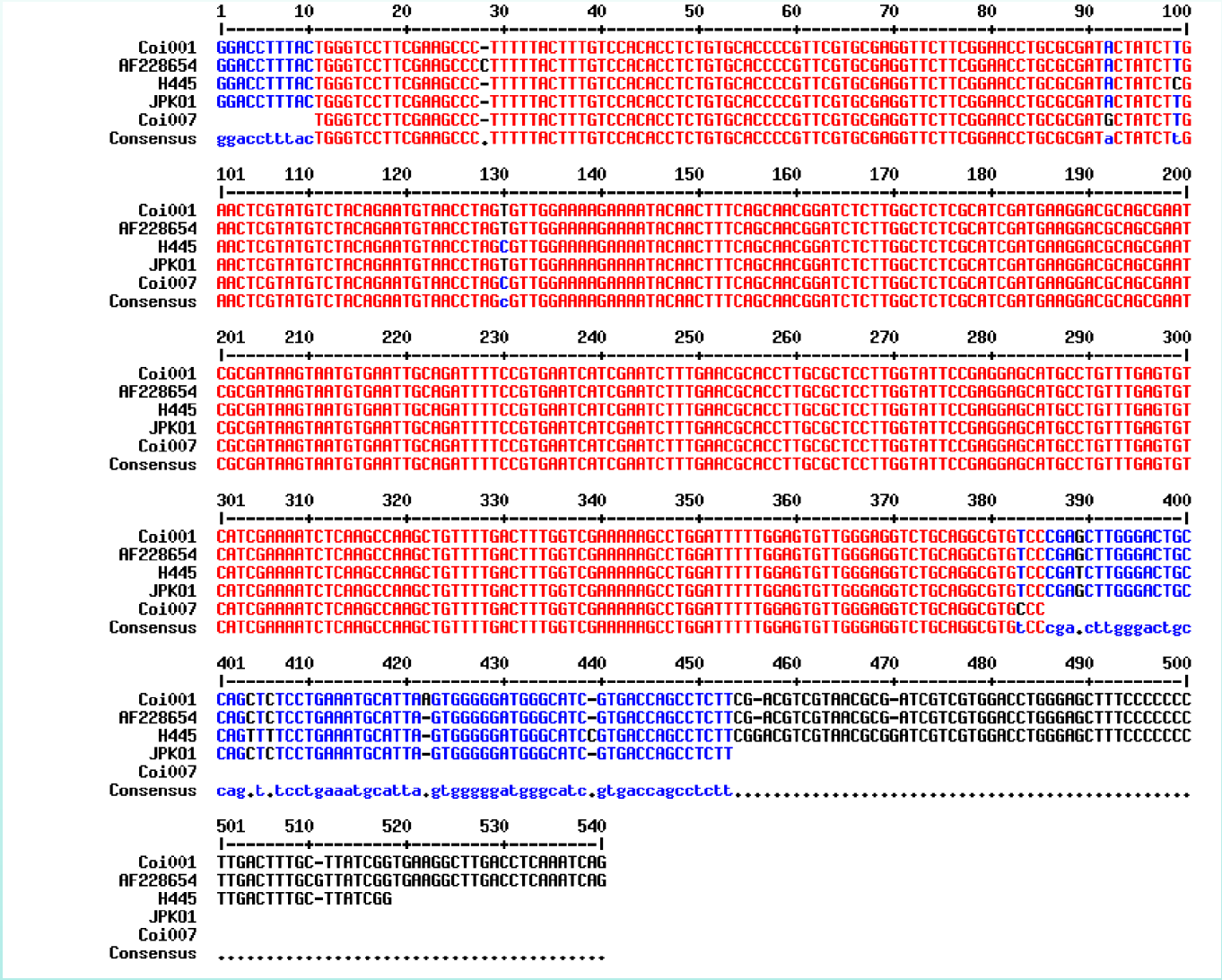
2 possible types of primers:



Sequencing



➢Molecular characterization of ectomycorrhizal Basidiomycetes



Molecular characterization of ectomycorrhiza

⇒ 600-700 bp amplicons of the ribosomal operon obtained from PCR using primers ITS 1F and ITS 4B (in 60% of ECM root tips samples analyzed)

⇒ Up to date, 68 ectomycorrhizal (ECM) apices were successfully analyzed for molecular characterization of the fungal partner

⇒ PCR also tested on DNA from sporophores (on-going sequencing of amplicons)

⇒ Molecular analyzes indicated the presence of different genera, in particular *Russula, Amanita, Boletus and Tomentella*

Conclusion

1) High diversity of ectomycorrhizal trees:

-> Lowland Dipterocarp forest (Sungai Telang; 2,6 ha) :
11 different species among 20 adult ECM trees

-> Mountain forest (Rimbo Candi; 2 x 500 m^2) :
11 different species among 26 adult ECM trees

2) Few fungi ectomycorrhizal or saprotrophic found in Lowland forest // Mountain forest => unfavorable season ?

3) More than 200 different morphotypes observed:
-> 1 to 8 different morphotypes/tree (4.8 at average)

4) The first results of molecular analyzes highlight the predominance of the genus *Tomentella* although no fruiting bodies observed so far.

General conclusion

-> High fungal diversity observed at this stage

-> ECM : essential key component to preserve and optimize in the framework of land rehabilitation, agroforestry & forest plantation programs using indigenous tree species

Perspectives

Diversity studies :

1- Diversity of ectomycorrhizal trees:

-> botanical identification from sapwood samples through anatomical observation & partial sequencing of the trnL chloroplast gene

2 - Diversity of ectomycorrhiza :

-> PCR analyses to be pursued : Dual characterization of the fungus (sequencing rDNA ITS region) & its host plant (sequencing trnL gene) from stored ECM root samples

-> Microsatellites : Study of the mycelial networks

Perspectives

Functional studies :

3- Carbon & Nitrogen transfers between mature trees and seedlings via the ECM mycelial network :

-> isotopic studies (natural abundance of leaves in ^{13}C and ^{15}N) to assess the role of ECM fungi in seedling regeneration (Rimbo Candi)

4 - Inoculation studies :

-> PCR analyses to be pursued : Dual characterization of the fungus (sequencing rDNA ITS region) and its host plant (sequencing trnL gene) from stored ECM root samples.

Terima kasih